The secret of rejuvenation relies on lysosome: a good listener for stressed and broken mitochondria

Author: Yi-Tang Lee, Marzia Savini, Guo Hu
Title of previewed paper: Mitochondrial translation and dynamics synergistically extend lifespan in *C. elegans* through HLH-30

Mitochondria is important for maintaining cellular energy homeostasis through metabolic programming and inter-organelle communication. These functions are highly regulated by the fusion/fission dynamic cycle of which alteration through age has been reported to be distinct in different tissues. For example, fragmented mitochondria were reported to be predominant in mechanosensory neurons of aged *C. elegans*[1], while fused mitochondria were observed in oocytes of aged mice[2]. Interestingly, mutant worms such as *daf-2* have been reported to require mitochondrial fusion GTPases for lifespan extension[3]. Conversely, intestinal mitochondrial fission confers lifespan benefits[4]. Functionally speaking, mitochondrial fission is crucial for selective mitophagy to preserve mitochondrial integrity, and it has been demonstrated to be crucial for selective removal of deleterious mtDNA in *Drosophila* germline[5]. On the other hand, mitochondrial fusion enables maximum oxidative phosphorylation through content mixing of partially damaged mitochondria into a functional network[6]. Even though mitochondrial dynamics is pivotal, other pathways such as mitochondrial translation and mitochondrial unfolded protein response (UPR_{mt}) play important roles in modulating mitochondrial function[7]. UPR_{mt} is activated upon disruptions of mitochondrial protein folding or function under mitochondrial stress[7]. In a 2013 study, *Houtkeeper et al*. discovered that alteration in mitochondrial translation level by either genetic manipulation or drug administration induces mitonuclear protein imbalance with subsequent UPR_{mt} activation to confer lifespan benefits[8]. In this same study, the authors noticed an alteration in mitochondrial dynamics in *C. elegans* with defective mitochondrial translation, in accordance with the observation by our group that long-lived mutants show changes in mitochondrial dynamics[4]. Lysosome, as another signaling hub, is also important for longevity regulation. In *C. elegans*, the TFEB ortholog *hlh-30* which regulates autophagosome and lysosome biogenesis is required for lifespan extension of some long-lived mutants[9]. Nevertheless, how mitochondria and lysosome interact to affect the aging progression is not well characterized. In this study, professor Houtkeeper and his group further dissected the underlying mechanism of how changes in mitochondrial dynamics and translation result in lysosomal and autophagy biogenesis to extend the lifespan of *C. elegans* synergistically[10].

The authors previously discovered the extension of *C. elegans* lifespan by knocking-down the mitochondrial ribosomal protein S5 (*mrsp-5*) triggers mitochondrial unfolded protein response (UPR_{mt}) that results in mitochondrial fragmentation. To further dissect the underlying mechanism, Liu *et al.* knocked-down *mrsp-5* together with the genes *eat-3* and *fzo-1,* involved in mitochondrial inner and outer membrane fusion, respectively. Using the reporter line targeting GFP to the mitochondrial matrix in muscles (myo-3p::GFP[mit]), *eat-3* or *fzo-1* RNAi significantly fragmented mitochondria at day2 and day7 of adulthood in both wild-type and *mrsp-5* animals, with a stronger effect in double RNAi conditions. Next, they tested a similar effect using the lifespan as a readout and observed a significant extension in double *mrps-5;eat-3* RNAi conditions compared to single *mrps-5* knockdown. Similarly, although *fzo-1* RNAi did not affect worm lifespan, double *mrps-5;fzo-1* RNAi resulted in longevity extension compared to single RNAi condition, suggesting that the longevity effect is mediated by the suppression of both mitochondrial fusion and translation.

Next, the authors examined whether the increase in stress response influences the longevity effect mediated by mitochondrial fragmentation. Using the *hsp-6* (mitochondrial HSP70) GFP

reporter line to monitor UPR^{mt}, *eat-3* or *fzo-1* RNAi resulted in UPR^{mt} activation response, whereas the *mrps-5;eat-3* or *mrps-5;fzo-1* double knockdown resulted in a stronger UPR^{mt} stimulation that is reduced upon aging. These results showed evidence of UPR^{mt} unique correlation with the longevity-mediated effect by the fragmented mitochondrial morphology and reduced mitochondrial translation. To test whether mitochondrial fragmentation is correlated with mitochondrial respiration, they analyzed oxygen consumption rate (OCR) at the basal level and maximum capacity upon FCCP treatment. Knocking-down *eat-3* or *fzo-1* respectively strongly decreased the maximum respiratory capacity without significantly affecting the basal respiration. In addition, basal respiration was further decreased upon *mrps-5;eat-3* but not upon *mrps-5;fzo-1* double RNAi compared to *mrps-5* RNAi-treated animals. Accordingly, no further reduction was assessed in maximum respiration upon *mrps-5;eat-3* or *mrps-5;fzo-1* double RNAi compared with *mrps-5* RNAi alone. Together, those results demonstrated a strong correlation of *mrps-5;eat-3* double RNAi not only with prolonging lifespan, but also suppressing mitochondrial respiration.

Since both mitochondrial fragmentation and translation promote longevity, Liu *et al.* decided to investigate the opposite event: mitochondrial fission phenotype with reduced mitochondrial translation. Surprisingly *drp-1* mutation further extends the longevity effect conferred by *mrps-5* RNAi. Simultaneous mutations in both *drp-1* and *fzo-1* were previously demonstrated to promote longevity as a consequence of a homeostatic equilibrium between fission and fusion cycles. Therefore, the same effect was tested on *mrps-5* RNAi using *drp-1;fzo-1* double mutants, which results in the suppression of *mrps-5* knockdown-mediated longevity. Next, UPR^{mt} activation was detected in the *drp-1;fzo-1* double mutant upon *mrps-5* abrogation, while no morphological change was reported. Together, these results suggested that UPR^{mt} activation is a common stress response upon mitochondrial translation inhibition, while in the same conditions the *drp-1;fzo-1* double mutants confer resistance to fragmentation.

To better understand how mitochondrial translation inhibition influences the mitochondrial network, a proteomic analysis was performed on worms fed by *mrps-5, mrps-5;eat-3* and *mrps-5;fzo-1* RNAi. Comparing *mrps-5* RNAi and its respective empty vector control, the GO analysis for cellular processes in down-regulated genes included “respiratory electron transport chain” and “positive regulation of grow rate”, while “mitochondrion organization” was enriched in up-regulated genes. To determine the common cellular processes that contribute to the synergistic effects of mitochondrial network and mitochondrial translation in longevity regulation, the authors compared each double RNAi-treatment to *mrps-5* RNAi alone. GO analysis for 46 common down-regulated proteins include “reproductive development, “sex differentiation” and “genitalia development”, while no significant GO terms were found in the 47 up-regulated proteins. To confirm those candidate cellular processes, Liu *et al.* first examined the reproduction capability of the longest-lived double *mrps-5;eat-3* RNAi animals, which results in a loss of fecundity. Second, because reproduction is tightly linked to the germline in canonical longevity pathways, the authors specifically knocked-down *mrps-5* and *eat-3* in the germline without observing lifespan extension previously observed in whole-body RNAi-treatment. Therefore, they tested *mrps-5;eat-3* RNAi on the lifespan of the germline-deficient *glp-1(e2141)* mutants, and found an additive effect in lifespan. Together, these results suggested that the germline is not the functional tissue for mediating longevity upon mitochondrial translation ablation.

To further dissect the downstream mechanism, the authors explored distinct mitochondrial electron transport chain (ETC) mediators, by testing their RNAi-mediated knockdown upon *mrps-5* RNAi in the *eat-3(tm1107)* or *drp-1(tm1108)* mutants. They discovered that only upon *hlh-30* inactivation, the longevity phenotype in both mutant backgrounds was suppressed, suggesting HLH-30’s role in mediating the synergistic longevity effects conferred by simultaneously suppressing mitochondrial translation and mitochondrial dynamics. To investigate HLH-30

localization, a GFP reporter line was employed. Individual *eat-3, drp-1* and *mrps-5* RNAi induced HLH-30 nuclear translocation, while a stronger nuclear enrichment was observed upon *mrps-5* knock-down in *eat-3(tm1107)* or *drp-1(tm1108)* mutants. Together, these data identified HLH-30 as the global downstream mediator of longevity mediated by inhibition of mitochondrial dynamics and mitochondrial translation.

Because of HLH-30's role in lysosomal biogenesis, an increase in the number of lysosomes was expected in those long-lived animals. To test this hypothesis, the authors assessed and confirmed an increase in GFP::LMP1 lysosomal marker in coelomocytes, as well as a reduction in enlarged lysosomes upon *mrps-5*;*eat-3* RNAi conditions compared to the empty vector control and to each individual RNAi treatment. Using electron microscopy, the authors observed an increase in overall number of lysosome-like structures indicated by both electron-lucent and electron-dense MVBs in the intestine and hypodermis of worms treated with both *mrps-5* and *eat-3* RNAi compared to the individual *mrps-5* RNAi. They further tested whether this increase in lysosome-like structures and MVBs was dependent on HLH-30 by analyzing *mrps-5* RNAi or *mrps-5;hlh-30* double RNAi in the *eat-3 (tm1107)* mutant background. The reduction in lysosome-like structures and MVBs was observed upon *mrps-5;hlh-30* double RNAi conditions, suggesting the role of HLH-30 in mediating lysosome biogenesis upon mitochondrial translation ablation.

Lastly, they determined whether an increase in the autophagy was induced in long-lived *msps-5;eat-3* double RNAi animals. Using the transgenic line expressing mCherry::GFP::LGG-1 dual reporter to monitor autophagy flux, Liu *et al.* observed an increase in auto-phagolysosome formation without affecting autophagosome levels in hypodermic cells, suggesting an elevated autophagic flux during mitochondrial translation and fusion inactivation. Overall, this paper described that upon both mitochondrial translation inhibition and mitochondrial fission or fusion condition, HLH-30 translocates into the nucleus to induce lysosomal biogenesis genes required to promote lifespan extension.

While many studies have shown that mild disturbance in the mitochondria delays the aging process and age-related dysfunctions in various animal models, the current research takes a step further to evaluate in detail the multi-facet of mitochondria functions in the context of aging using *C. elegans*. The observation that perturbed mitochondrial translation and increased mitochondrial fragmentation synergistically promote lifespan extension indicates a tight link between these two processes. But the detailed molecular machinery bridging these two mitochondrial functions is still unclear.

Upon mitochondrial translation inhibition, it has been observed that increased UPR_{MT} is concurrent with lifespan extension[8]. However, blocking UPR_{MT} did not hamper mitochondrial translation inhibition-related longevity, which suggests this lifespan extension phenotype is independent from it. Interestingly, the authors found that increased nuclear localization of HLH-30 is essential for the lifespan benefits conferred by changing mitochondrial dynamics and the inhibition of mitochondrial translation. Although previous studies showed evidence on direct contact between mitochondria and lysosome via Rab-7 (Wong et al. in 2018), here the authors did not observe a physical interaction. Then, what is intriguing is how mitochondrial translation inhibition activates HLH-30. One possibility includes metabolites produced during the mitochondria stress that signal into the nucleus and activate the transcription of HLH-30. Alternatively, mitochondrial signals indirectly dephosphorylate cytosolic HLH-30 by activating an intermediate key player such as a phosphatase allowing HLH-30 to translocate into the nucleus. On another note, it has been shown that increase in autophagy can be mediated by mTOR inhibition or AMPK activation, then how autophagy contributes to longevity in the context of mitochondrial translation inhibition is fascinating. The clearance of damaged mitochondria

through mitophagy is critical in maintaining proper mitochondria functions in the stressed cells. Also an increase in lysosomal hydrolases activity may promote autophagic clearance, which conversely lead to lysosomal storage diseases. Thus, further investigation of autophagosome content and HLH-30 targeted genes may reveal precise functions of the lysosome in the context of mitochondrial translation inhibition-promoted longevity. Finally, tissue specific phenotypes induced by mitochondrial translation inhibition suggest a cell non-autonomous regulation and that further studies might identify metabolites that originate from mitochondria and uptake in intestine and hypodermis to modulate lysosomal biogenesis.

In this work, the authors carefully demonstrated the connection between the mitochondrial dynamics, mitochondrial translation, and lysosome biogenesis in the context of longevity using model organism *C. elegans*. Further studies are needed to illustrate how mitochondria stress transmits to the lysosomes and whether it is through certain metabolites that enable a cell non-autonomous regulation to achieve longevity. Based on these findings, targeting mitochondria-lysosome interaction may advance the therapeutic interventions in aging.

References.

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